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Determination of Monod Kinetics of Toxic Compounds by Respirometry for Structure-Biodegradability Relationships

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16. ABSTRACT

The key to the evaluation of the fate of toxic organic chemicals in the environment is dependant on evaluating their susceptibility to biodegradation. Biodegradation is one of the most important mechanisms in controlling the concentration of chemicals in an aquatic system because toxic pollutants can be mineralized and rendered harmless. Experiments using an electrolytic respirometer have been conducted to collect oxygen consumption data of toxic compounds from the list of RCRA and RCRA land banned chemicals (phenols and phthalates). The estimation of Monod kinetic parameters were obtained for all the compounds by a graphical method. The first order kinetic constants for the substituted phenols were related to the structure of the compounds by the group contribution method.

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Chapter 9

Determination of Monod Kinetics of Toxic Compounds by Respirometry for Structure–Biodegradability Relationships

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The key to the evaluation of the fate of toxic organic chemicals in the environment is dependant on evaluating their susceptibility to biodegradation. Biodegradation is one of the most important mechanisms in controlling the concentration of chemicals in an aquatic system because toxic pollutants can be mineralized and rendered harmless. Experiments using an electrolytic respirometer have been conducted to collect oxygen consumption data of toxic compounds from the list of RCRA and RCRA land banned chemicals (phenols and phthalates). The estimation of Monod kinetic parameters were obtained for all the compounds by a graphical method. The first order kinetic constants for the substituted phenols were related to the structure of the compounds by the group contribution method.

It has been estimated that 50,000 organic chemicals are commercially produced in the United States and a large number of new organic chemicals are added to the production list each year (1). The presence of many of these chemicals in the environment could be attributed to inadequate disposal techniques.

Since many of these hazardous chemicals can be detected in wastewater, their fate in wastewater treatment system is of great interest. Of many factors that affect the fate of these compounds, microbial degradation is probably the most important. Biodegradation can eliminate hazardous compounds by biotransforming them into innocuous forms, degrading them

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by mineralization to carbon dioxide and water. Prediction of the concentration of toxic chemicals in natural and engineered environments and assessing their effects on human and other species due to possible exposure requires information on the kinetics of biodegradation.

Howard et al. (2) have reviewed the different techniques for measuring kinetics of biodegradation. Due to the large number of chemicals, it is imperative that the method selected should not be labor intensive and time consuming. Also, the parameters obtained should be intrinsic; that is, dependant only on the nature of the compound and the degrading microbial community and not on the reactor system used for data collection. If this condition is satisfied, then the parameters obtained can be used for any reactor configuration and can be used in mathematical models to estimate the fate of the toxic chemicals.

It is possible to obtain intrinsic kinetic parameters from batch experiments for a single substrate, provided that the initial conditions are precisely known (3,4). Gaudy et al. (5) have shown that it is possible to use oxygen consumption to determine substrate removal because oxygen utilization during microbial energy production. So, it is possible to obtain intrinsic kinetic parameters from oxygen consumption data from single batch experiments. Grady et al. (6) also demonstrated that the values of the parameters obtained from oxygen uptake are in general agreement with values determined by more traditional methods.

The measurement of oxygen consumption is one of the oldest means of assessing biodegradability, and respirometry is one technique for measuring oxygen consumption. In respirometric methods substrate samples, along with biomass, are kept in contact with a gaseous source of oxygen. Oxygen uptake by microorganisms over a period of time is then measured by change in volume or pressure of the gas phase. An alkali is included in the apparatus to absorb carbon dioxide produced during biodegradation. Samples are usually incubated at constant temperature and are kept away from light, but the procedures vary with different instruments.

Electrolytic respirometry is the most commonly employed method for biodegradation studies. Generally it is used for biochemical oxygen demand (BOD) determination with the exception of studies by Dojlido (7), Oshima et al. (8), Gaudy et al. (9) and Grady et al. (6), who used it for determination of biodegradation kinetics. The data collection is automatic with electrolytic respirometry, so manual collection and analysis of samples is not required to follow the course of substrate degradation. Since the respirometer can be automated, the length of the experiment is less important and thus unacclimated biomass can be used in the experiments.

The objective of this study was to find degradation rates of the Resource Conservation and Recovery Act

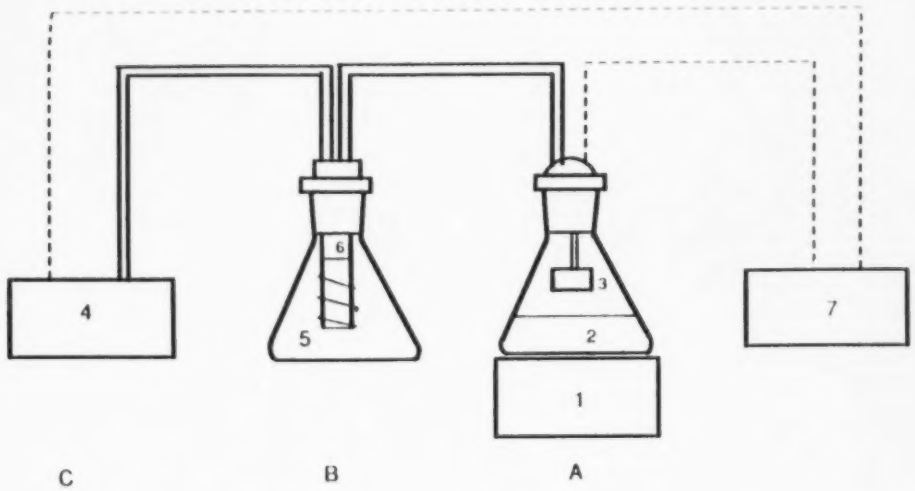
(RCRA) and RCRA land banned compounds with the use of mixed cultures obtained from a wastewater treatment plant, so that the rates obtained in the laboratory could be used to predict degradation rates in the environment. Chemicals were obtained from Aldrich Chemical Company (Milwaukee, WI) and were reported 99+% pure. The phenols and phthalates tested were phenol, o-cresol, m-cresol, p-cresol, 2,4-dimethyl phenol, dimethyl phthalate, diethyl phthalate, dipropyl phthalate, dibutyl phthalate and butyl benzyl phthalate.

Experimentation

Electrolytic respirometry studies were conducted using an automated continuous oxygen measuring Voith Sapromat B-12 electrolytic respirometer (Voith-Morden, Milwaukee, WI). This system consists of a temperature controlled water bath which contains the measuring units, a recorder for digital indication, a plotter for direct presentation of the oxygen uptake curves of substrates, and a cooling unit for conditioning and continuous recirculation of water bath contents. The system used had 12 measuring units each connected to the recorder. Each unit, as shown in Figure 1, consisted of a reaction vessel A, with a carbon dioxide absorber (soda lime) mounted in a stopper, an oxygen generator B, and a pressure indicator C. Interconnected by hoses, the vessels form a sealed measuring system so that barometric pressure fluctuations do not adversely affect the results. The magnetic stirrer in the sample to be analyzed provides vigorous agitation, thus ensuring effective gas exchange. Microorganism activity in the sample creates a pressure reduction that is recorded by the pressure indicator which controls both the electrolytic oxygen generation and plotting of the measured values. The CO_2 generated is absorbed by soda lime.

The N_2/O_2 ratio in the gas phase above the sample is maintained constant throughout the experiment by an on/off feedback control loop. As oxygen is depleted, pressure reduction is created in the sample and, as a result, the level of the sulphuric acid in the pressure indicator rises and comes in contact with a platinum electrode. This completes the circuit and triggers the generation of oxygen by electrolysis. The electrolytic cell provides the required amount of oxygen to the reaction vessel by electrolytic dissociation of a $CuSO_4$-H_2SO_4 solution thus alleviating the negative pressure. As the level of the electrolyte in the pressure indicator decreases, the contact with the electrode is broken. This switches off the electrolytic cell. The amount of oxygen supplied to the sample is recorded directly in milligrams per liter by the recorder. The recorder is connected to a microcomputer which records data from the measuring units every 15 minutes.

The nutrient solution is made as per Organization of



A. Reaction vessel.

B. Oxygen generator.

C. Pressure indicator.

1. Magnetic stirrer.
2. Sample (250 ml)
3. Carbon dioxide absorber
4. Pressure indicator
5. Electrolyte.
6. Electrodes
7. recorder.

Figure 1. Schematic of a Measuring Unit

Economic Cooperation and Development (OECD) guidelines (10). It contains 10 ml of solution A and 1 ml of each of the solutions B to F per litre of synthetic medium : solution A - KH_2PO_4 8.5 g, K_2HPO_4 21.75 g, $Na_2HPO_4.2H_2O$ 33.4 g, and NH_4Cl 2.5 g; solution B - $MgSO_4.7H_2O$ 22.5 g; solution C - $CaCl_2$ 27.5 g; solution D - $FeCl_3.6H_2O$ 0.25 g; solution E - $MnSO_4.4H_2O$ 39.9 mg, H_3BO_3 57.2 mg, $ZnSO_4.7H_2O$ 42.8 mg, $(NH_4)_6Mo_7O_{24}$ 34.7 mg and $FeCl_3$.EDTA 100 mg; and solution F - Yeast extract 150 mg. The chemicals for each of the solutions A, B, C, D, E and F, are dissolved in 1000 ml of deionized water. The solution D is freshly prepared immediately before the start of an experiment.

The microbial inoculum was an activated sludge sample from The Little Miami wastewater treatment plant in Cincinnati, Ohio, which receives predominantly domestic sewage. The sludge was allowed to settle for about an hour, decanted, and then aerated at room temperature for 24 hours. The dry weight of sludge was determined by drying samples, in duplicates, of 1 ml, 2 ml and 3 ml at 105°C overnight. A concentration of 30 mg/l of sludge as dry matter was used in the experiment. The total volume of the synthetic medium in the 500 ml capacity reactor vessels was brought up to a final volume of 250 ml.

The concentration of the test compounds was 100 mg/l of medium. Aniline was used as the biodegradable reference compound at a concentration of 100 mg/l. The stock solutions were made in distilled water for aniline and phenol with concentration of 5 g/l. Other compounds were added directly to the reaction vessels using microliter syringes.

The typical experimental system consisted of duplicate flasks for the reference substance aniline and the test compounds and a single flask for toxicity control (test compound + aniline at 100 mg/l) and an inoculum control. The contents of the reaction vessels were stirred for an hour to ensure a steady state of the endogenous respiration at the initiation of oxygen uptake measurements. Then the test compound and aniline were added. The reaction vessels were incubated at 25°C in the temperature controlled water bath and stirred continuously throughout the run. The microbiota of the activated sludge used as inoculum were not preacclimated to the test compounds. The incubation period of the experimental run was between 20 to 40 days.

Biokinetic Parameters

Although many models have been proposed for microbial growth and substrate removal, the Monod relationship is among the most popular kinetic expressions used today. The Monod relation, in combination with the linear law for substrate removal, can provide an adequate description of microbial growth behavior. It states that

the rate of cell growth is first order with respect to biomass concentration (X) and of mixed order with respect to substrate concentration (S).

$$dX/dt = (S\mu_m X)/(K_s + S) \quad \ldots\ldots\ldots (1)$$

Cell growth is related to substrate removal by the linear law

$$dX/dt = - Y\ (dS/dt) \quad \ldots\ldots\ldots\ldots (2)$$

The kinetic parameters of interest are the maximum specific growth rate μ_m, the half saturation constant K_s, and the yield coefficient Y. Here these kinetic parameters were estimated directly from the experimental oxygen uptake curves. The yield coefficient Y and the half saturation constant K_s were determined by the method of Grady et al. (6) and the maximum specific rate constant, μ_m, was determined by the method of Gaudy et al. (10). If the concentration of substrate and biomass are expressed in oxygen equivalents, then the oxygen uptake O_u, at any time in a batch reactor may be calculated from :

$$O_u = (S_o - S) - (X - X_o) \quad \ldots\ldots\ldots\ldots (3)$$

where subscript 'o' denotes initial condition.

The yield coefficient Y was estimated from the oxygen consumption at the beginning of the plateau of the oxygen uptake curve by :

$$Y = (1 - O_{up}/S_o) - Y_p \quad \ldots\ldots\ldots\ldots\ldots (4)$$

where Y_p is the product yield and O_{up} is the cumulative oxygen uptake value at the initiation of the plateau.

In this study the product yield was negligible. The oxygen uptake has reached its plateau when the cumulative oxygen uptake value is approximately constant over a long period of time. At this plateau, oxygen uptake is due to endogenous respiration of the microbiota rather than the degradation of the test substrate.

If the assumption that $S>>K_s$ is made, then the term $S/(K_s + S)$ in Equation 1 approaches one and simplifies the equation. Combining this simplified equation with Equation 2 and integrating and substituting in Equation 3 gives

$$\ln [X_o + O_u/(1/Y - 1)] = \ln(X_o) + \mu_m t \quad \ldots (5)$$

The plot of $\ln[X_o + O_u/(1/Y - 1)]$ versus time will give a straight line with slope μ_m. The oxygen uptake value O_u' associated with one-half of the estimate of μ_m is used in

$$K_s = S_o - O_u'/(1 - Y) \quad \ldots\ldots\ldots\ldots\ldots (6)$$

to get the estimate of K_s. The oxygen uptake curve before the plateau is utilized to get this estimate of K_s. Note that Equation 5 is valid only in the initial period of degradation when $S>>K_s$ and not throughout the degradation period.

Structure-Activity Relationships

Predictive techniques, based on relationship between structure and biodegradability, are important due to the increasing number and type of chemicals entering the environment and the fact that assessing biodegradability may be time consuming and expensive.

In the field of biodegradation there are several studies which have attempted to correlate some physical, chemical or structural property of a chemical with its biodegradation. Based on the type and the location of the substituent groups, Geating (11) developed an algorithm to predict biodegradation. Qualitative relationships for different compounds have been investigated by others, but quantification is required for regulatory purposes.

First order rate constants of biodegradation, or five day BOD values for chemicals, have been correlated using both physical and chemical properties. Paris, et al. (12,13) established a correlation between second order biodegradation rate constants and the van der Waal's radius of substituent groups, for substituted anilines and for a series of para-substituted phenols. Wolfe, et al. (14) correlated second order alkaline hydrolysis rate constants and biodegradation rate constants for selected pesticides and phthalate esters. Several workers have observed a correlation between biodegradability and lipophilicity, specifically octanol/water partition coefficients (log P). Paris, et al. (15) found a good correlation between biodegradation rate constants and log P, for a series of esters of 2,4-dichlorophenoxy acetic acid. Banerjee, et al. (16) obtained a similar relationship for chlorophenols. Vaishnav, et al. (17) correlated biodegradation of 17 alcohols and 11 ketones with octanol-water coefficients using 5-day BOD data. Pitter (18) found a dependence of biodegradation rates on electronic factors, like the Hammett substituent factors, for a series of anilines and phenols. Dearden and Nicholson (19) have correlated 5-day BOD values with modulus differences of atomic charge across a selected bond in a molecule for amines, phenols, aldehydes, carboxylic acids, halogenated hydrocarbons and amino acids. A direct correlation between the biodegradability rate constant and the molecular structure of the chemical has been used by Govind (20) to relate the first order biodegradation rate constant with the first order molecular connectivity index and by Boethling (21) to correlate the biodegradation rate constants with the molecular connectivities for esters, carbamates, ethers, phthalates and acids.

Group Contribution Approach

This approach is widely used in chemical engineering thermodynamics to assess the variety of pure component properties such as liquid densities, heat capacities and critical constants for organic compounds. This method is similar to the Free-Wilson model, which is widely used in pharmacology and medicinal chemistry. Using a group contribution approach, a large number of chemicals of interest can be constituted from perhaps a few hundred functional groups. Property prediction is based on the structure of the compound. According to this method, the molecules of a compound are structurally decomposed into functional groups or their fragments, each having unique contribution towards the compound properties.

The biodegradability rate constant, k, is expressed as a series function of contributions, α_j, of each group of the compound. The first order approximation of this series function representing biodegradation rate constant can be expressed as

$$\ln (k) = \sum_{j=1}^{L} N_j \alpha_j \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

where N_j is the number of groups of type j in the compound, α_j is the contribution of group of type j and L is the total number of groups in the compound.

Using Equation 7 for each compound, a linear equation is constructed. For a given data set this generates a series of linear equations which are solved for α's using linear regression analysis.

Results and Discussion

Oxygen uptake curves for the test compounds, reference compound aniline, the toxicity control (aniline + test compound) and the endogenous control systems were generated over a period of 20 to 40 days. Within a period of 10 days all the controls, test compounds and aniline revealed the lag phase, biodegradation phase and the plateau region. Figures 2 and 3 illustrate the representative oxygen uptake curves generated for two of the test compounds, phenol and dibutyl phthalate.

These data reveal that all the test compounds, namely phenol, o-cresol, m-cresol, p-cresol, 2,4-dimethyl phenol, dimethyl phthalate, diethyl phthalate, dipropyl phthalate, dibutyl phthalate and butyl benzyl phthalate were biodegradable at the concentration level of 100 mg/l under the experimental conditions. According to OECD (11), the results of the degradation experiment were valid because 60% degradation of control substance aniline was achieved within a period of 28 days. Within a period of 40 days all the compounds were degraded between 80% - 95%.

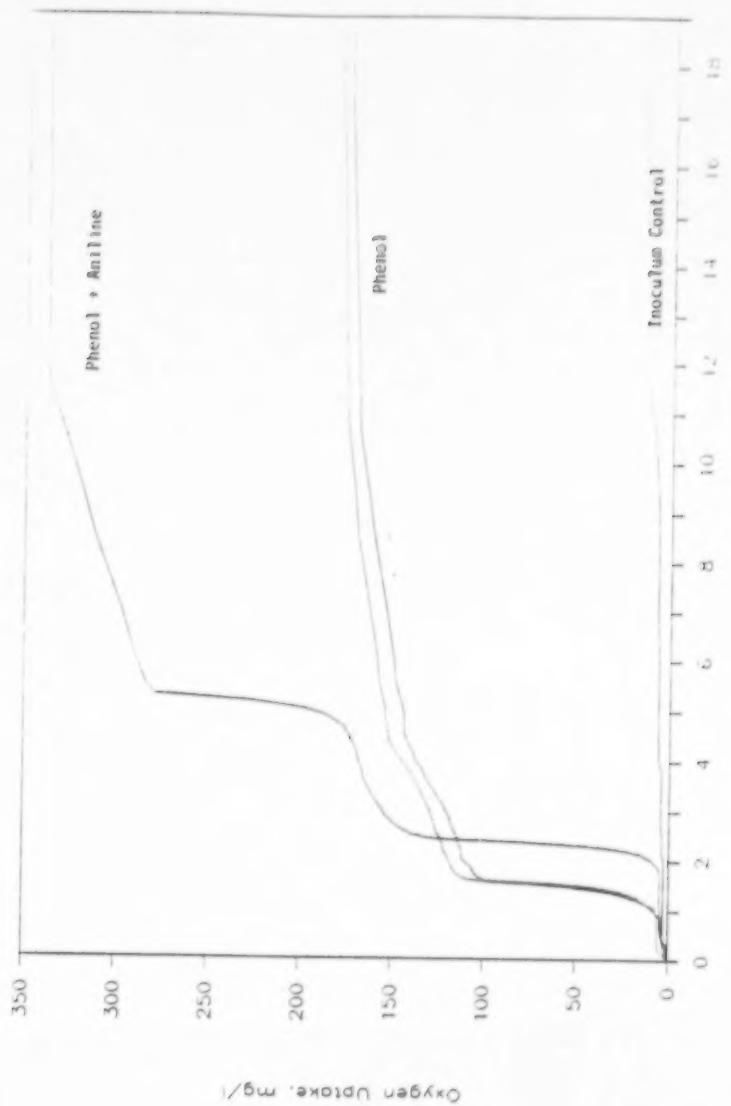


Figure 2. Oxygen Uptake of Phenol

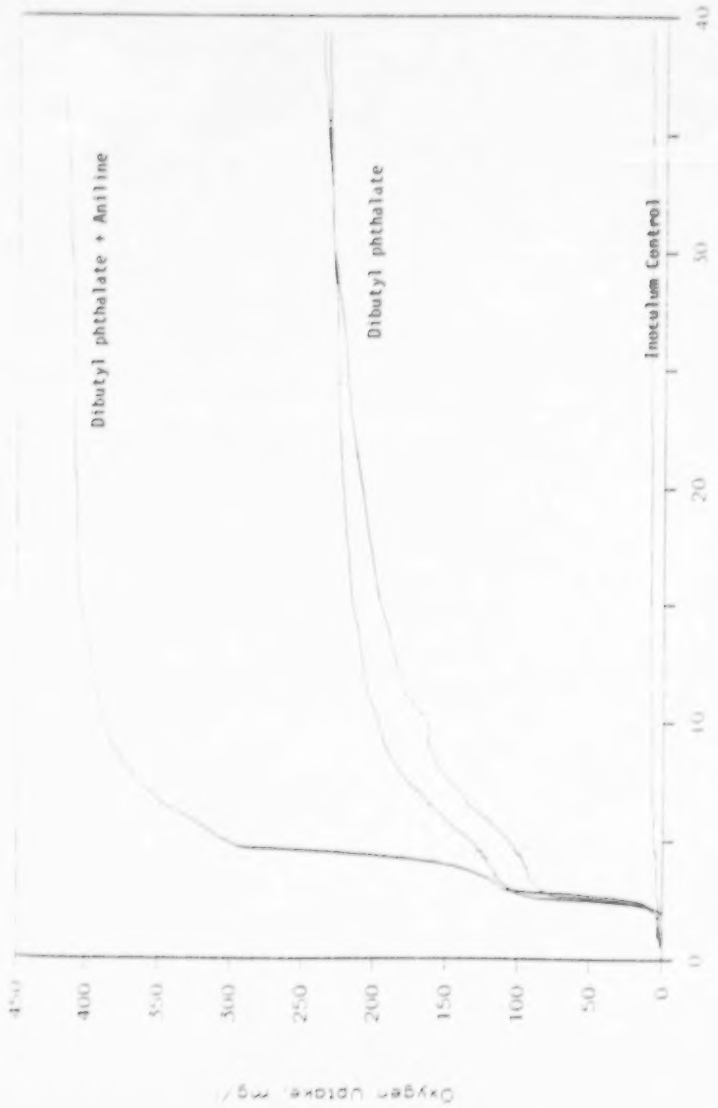


Figure 3. Oxygen Uptake of Dibutyl Phthalate

The toxicity control data (test compound + aniline) revealed that, except butyl benzyl phthalate, none of the test compounds were inhibitory towards the biodegradation of aniline in the sludge. Butyl benzyl phthalate causes a slight inhibitory effect on aniline degradation by increasing the acclimation time. The oxygen uptake curves of all the toxicity controls demonstrated two consecutive degradation phases, one for the test compound and another for positive control compound aniline as shown in Figure 2 and 3, and this behavior is characteristic for cultures in which the test compound is not inhibiting the biodegradation of the control substrate. The difference between the total oxygen uptake of the toxicity control (test compound + aniline), and of the corresponding test compound, was approximately equal to the total oxygen uptake of the control compound aniline.

Estimates of the Monod kinetic parameters, and the maximum specific substrate uptake rate per unit biomass, k_m ($=\mu_m/Y$) for phenols, phthalates and aniline, obtained by the method described before, are given in Table I.

Table I. Estimate of Monod Parameters

Compound	k_m (day^{-1})	Y	μ_m (day^{-1})	K_s (mg/l)
Aniline	16.1	0.38	6.15	6.10
PHENOLS				
Phenol	16.9	0.58	9.82	9.43
o-Cresol	10.0	0.41	4.10	16.41
m-Cresol	17.3	0.46	7.97	17.62
p-Cresol	18.5	0.33	6.11	27.78
2,4-Dimethyl phenol	14.4	0.39	5.62	14.07
PHTHALATES				
Dimethyl phthalate	16.4	0.43	7.07	41.68
Diethyl phthalate	4.5	0.46	3.00	11.67
Dipropyl phthalate	12.0	0.48	5.78	15.81
Dibutyl phthalate	12.0	0.58	6.95	51.38
Butyl benzyl phthalate	12.8	0.61	7.80	36.25

The measured rate constants suggest that, in comparison to phenol, the presence of an o- or p-methyl group renders a phenolic compound less biodegradable. Substituents in the para position have a less pronounced effect than in the ortho position. This also explains the μ_m value of 2,4-dimethyl phenol which is smaller than the one for p-cresol but greater than the one for o-cresol.

The introduction of a m-methyl group seemed to have little effect on the biodegradability of the phenolic

compound. Sugatt et al. (22) have reported that the low molecular weight phthalates degrade slightly faster than the high molecular weight ones, but no such trend was observed in this study. Comparison of our results with those reported in the literature (22,23,24,25) was not possible because either the rates were not reported, or they were second order rates with different units. Our values for half saturation constant K_s, are high for some of the compounds. This result might be caused by the linearized graphical approach used for its estimation.

It is necessary to have at least five data points for each unknown variable to get a statistically valid correlation. So it is imperative to ensure that each group for which a contribution is calculated occurs in at least five compounds. The contributions of different groups were calculated from literature data (26). These data were first order constants calculated by the equation :

$$dBOD/dt = k\ BOD \quad \text{.....(8)}$$

The groups and their contribution parameters are given in Table II. The data set used to calculate contribution parameters did not include the cresols, phenol and 2,4-dimethyl phenol. The contribution of the phthalate group was not calculated because either not enough data were available or the only data available were for those phthalates used in this study.

Table II. Group Contribution Parameters

No.	Group		α_j
1	Methyl	CH_3	-3.460
2	Hydroxy	OH	-2.983
3	Aromatic CH	ACH	-0.8340
4	Aromatic C	AC	1.9730

Using the contribution parameters of Table II, first order rate constants were predicted for phenols. The comparison between the predicted values and the values obtained from experimental data, using Equation 8 are given in Table III. The predicted values are within 10% of the experimental values. However, the method described is a first order approximation which assumes that there are no group interactions. Additional studies are needed to verify this assumption.

Conclusion

Based on the oxygen uptake data all the tested phenols and phthalates are biodegradable in activated sludge with

our experimental conditions. Similar results have been noted in the literature. All compounds were degraded in excess of 80%. A methyl group in o- or p- position of phenolic compounds decreases the degradation rate in comparison to phenol, while a m-methyl group does not affect the degradation rate. No such trend was observed with phthalates. With the exception of butyl benzyl phthalate, none of the test compounds inhibit aniline degradation. More data are required to determine whether or not unique group contributions exist with respect to its position (o, m, and p), and to include more groups in the structure-activity relationships.

Table III. Comparison of Actual and Predicted ln(k) Values

Compound	Actual ln(k)	Predicted ln(k)	% Error
o-Cresol	-6.0890	-5.8330	4.20
m-Cresol	-5.7706	-5.8330	-1.08
p-Cresol	-5.8659	-5.8330	0.56
2,4-Dimethyl Phenol	-6.2472	-6.4860	-3.82
Phenol	-5.6891	-5.1800	8.95

Nomenclature

k	first order biodegradation constant (hr^{-1})
k_m	maximum specific substrate uptake rate per unit biomass (day^{-1})
K_s	half saturation constant (mg/l)
L	total number of groups
N_j	number of groups of type j
O_u	oxygen uptake (mg/l)
O_{up}	oxygen uptake at the plateau (mg/l)
S	substrate concentration (mg/l)
X	biomass concentration (mg/l)
Y	biomass yield coefficient
Y_p	product yield coefficient
α_j	contribution of j th group
μ_m	maximum specific rate constant (day^{-1})

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